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Effects of Purified Pentachlorophenol on the Serum Proteins of Young Pigs

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Diverse industrial and agricultural applications of the pesticide pentachlorophenol (PCP) results in the direct or indirect exposure of a large fraction of farm animals and humans alike. Depending on the mode of PCP application, exposure can be by inhalation, ingestion or dermal absorption. Human occupational and accidental poisonings result in many nonspecific symptoms which can ultimately lead to coma and even death (MENON, 1958; ROBSON, et al., 1969). Domestic animals exposed to PCP frequently suffer from skin lesions, exhibit poor weight gains and have a general unhealthy appearance (THOMAS et al., 1977). Post-mortem examinations have not been able to reveal any definite pathological effects which could be specifically attributed to PCP (BENEVUE and BECKMAN, 1967). The apparent absence of a specific target of PCP toxicity suggests that PCP is nonspecific in its action. If the hematopoietic and/or lymphoid systems are affected, individuals will be more vulnerable to disease, infection, and malignancy. Consequently a reduced immunological reactivity might be responsible for the unhealthy appearance and poor weight gains of PCP-exposed animals.

A series of investigations were initiated by GREICHUS et al., (1979) to determine what clinical signs might indicate overt PCP poisoning. These studies investigated the effects of purified PCP on young pigs. This study is a companion investigation detailing the influence of pure PCP on leucocytes, serum proteins, and IgG of young pigs. These parameters were chosen to indicate if chronic PCP exposure could induce sufficient immunosuppression to be a potential health hazard.

Materials and Methods

Six, 6-week old pigs were randomly assigned to each of four treatment groups. Three groups received a gelatin capsule containing a mixture of lactose and either 5, 10, or 15 mg/kg body weight 95% pure PCP (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin) daily for 30 days. The control group received a similar capsule containing an equivalent amount of lactose only. Food and water was administered ad libitum. All pigs were weighed upon the initiation of treatment and at weekly intervals thereafter (days 0, 8, 15, 22 and 30). Five milliliter blood samples were taken on days 0, 15 and 30 for total leucocyte counts, serum protein profiles and IgG determinations.

Total leucocyte counts were determined by standard methods using a Model F Coulter Counter. Serum protein profiles were determined by cellulose acetate electrophoresis using Titan III (Helena Laboratories, Beaumont, Texas) plates and Tris-Barbital pH 8.8 buffer (Gelman High Resolution Buffer) for 30 minutes using constant current and an initial potential of 180 volts. After electrophoresis the plates were stained with Ponceau S, destained with 5% acetic acid and dehydrated in methanol. After clearing and scanning at 525 nm with a Gilford 2400 Spectrophotometer, densitometric quantitation was performed by planimetry. IgG determinations were made by electroimmunodiffusion using a modification of the procedure used by MERRILL et al., (1967). Rabbit anti-porcine IgG (1:150 v/v final dilution) was incorporated in 0.75% w/v agarose in 0.05M barbital buffer pH 8.8 and poured between two 5 X 7.5 cm glass slides separated by 3 layers of Scotch No. 33 electrical tape. An aliquot of 0.4 uL of diluted or undiluted serum was loaded into holes punched by a 17 gauge needle and electrophoresed at 120 volts (about 1 mA/cm) for 200 min. at 4°C. Measurement of precipitin peak areas was made using a Peak Height and Area Estimator (Bio Rad Laboratories, Richmond, California). The area of the precipitin peaks were proportional to the amount of IgG present. Quantitation of the IgG was determined using a calibration curve made with known amounts of porcine IgG (Cappel Laboratories, Cooper Diagnostics, Inc., West Chester, Pennsylvania). This technique enabled the accurate and reproducible detection of 1.5 ± 0.4 ug IgG (mean ± SD) which was equivalent to 0.38 ± 0.1 mg/ml. The standard error of the measurement was 6.7 ± 0.4%.

All statistical analyses were performed using one way analysis of variance in conjunction with the Dunnett's test. Single tailed comparisons were made and only those with $p<0.05$ or $p<0.01$ were considered significant.

RESULTS AND DISCUSSION

GREICHUS et al., (1979) have reported the results of blood chemistries; PCP concentrations in the liver, kidney, spleen, brain, muscle and plasma; weight gains; organ to body weight ratios of kidney and liver; and tissue histopathologies of the pigs used in this study. They report that pigs treated with PCP showed no overt signs of toxicosis other than enlarged livers and higher levels of blood urea nitrogen than did the controls.

Recent investigations on the immunotoxicity of environmental pollutants have revealed that the hematopoietic and lymphoid systems are frequently more sensitive indicators of toxicity than the previously used clinical parameters (SHARMA, 1981). Broad indicators of immunological reactivity, i.e., white blood cell counts, serum protein profiles and serum IgG concentrations were therefore examined in this study as possible indicators of PCP toxicity.

There were no significant differences in the number of total white blood cells among any of the groups at the onset of this study.

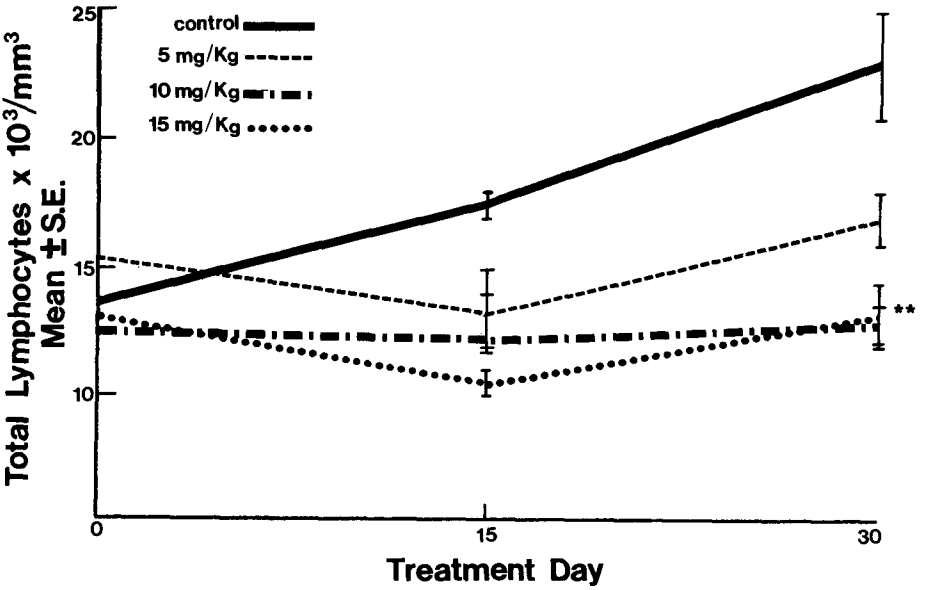

Figure 1. Total leucocyte determinations of PCP-treated and control pigs at initiation, midpoint and termination of treatment. Each point represents the mean ± standard error of six pigs ** indicates $p < .01$.

Leucocyte counts in the control group were observed to increase continuously throughout the duration of the experiment (FIG. 1). The initial 27% increase during the first half of the study and overall 94% increase reflects a normal pattern of immunological hematopoietic maturation. In contrast all PCP treatment groups experienced a 3 to 20% reduction in total leucocytes after the initiation of treatment. By the termination of this investigation the hematopoietic system appeared to be recovering. Overall, on day 30, there was a slight (6-19%) increase in the total leucocytes of all treatment groups; however this increase was significantly less than that of the control group (FIG. 1). No differential counts were made to determine which, if any, of the various populations of white blood cells were most affected.

Cellulose acetate electrophoretic profiles revealed that of the five major electrophoretic fractions only the relative proportion of the gamma globulin fraction was altered by PCP ingestion (FIG. 2). This fraction is important for the maintenance of health since in addition to serum antibodies, it also contains a bacteriolytic enzyme, lysozyme, and various proteins involved in the complement system. An increase, analogous to that seen in total

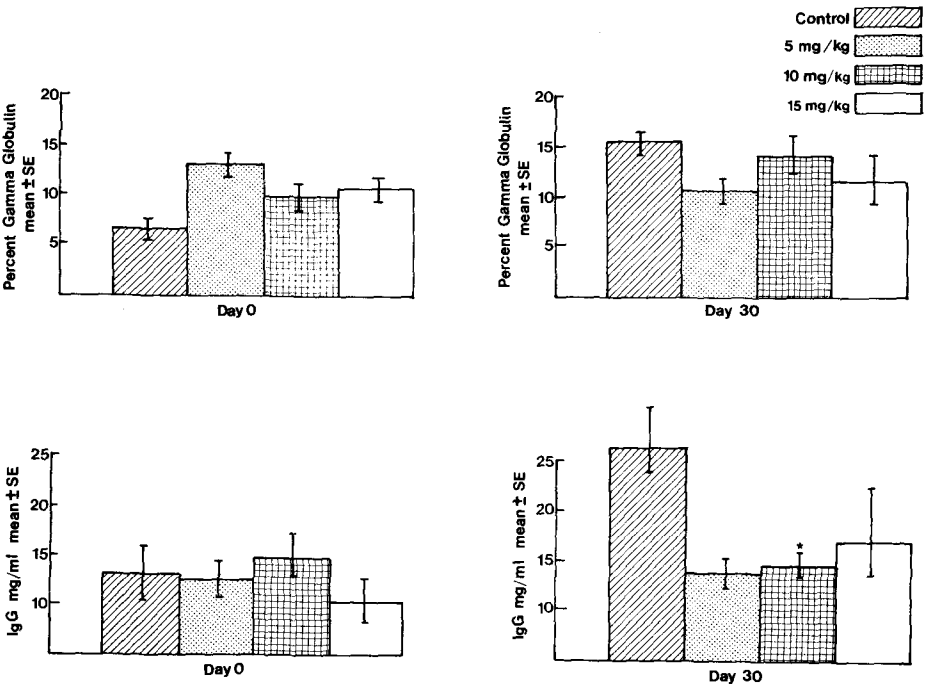

Figure 2. Percentage gamma globulin and IgG concentration in the serum of PCP-treated and control pigs. Each bar represents the mean ± standard error of six pigs and * indicates $p < .05$.

leucocytes, was observed in the gamma globulin fraction of the control group. This increase was significantly greater than the increases observed in the treatment groups.

Since IgG is the predominant serum and intravascular antibody, is capable of crossing the placenta and protecting a fetus, and is usually the predominant protein in the gamma globulin fraction, it was quantitated by electroimmunodiffusion and used as an additional indicator of immunosuppression. For the control group, the increase in IgG directly paralleled the increase in gamma globulin (FIG. 2). The 5 mg/kg and 10 mg/kg groups, however, showed only a slight IgG increase which was significantly less than that of the controls. An unexpected observation was the comparatively greater concentration of IgG in the serum of the 15 mg/kg group than in either of the other treatment groups receiving lower doses of PCP. If PCP is more cytotoxic for lymphocytes than for other populations of white blood cells, the concentration of PCP in the tissues and blood of this high treatment group may have been suffi-

ciently high to lyse the lymphocytes. Had this happened, a release of intracellular IgG would have occurred and subsequently raised the IgG concentration higher than would normally result from ordinary secretory processes.

Table 1. Percent Change[a] in Total Leucocytes, Gamma Globulin and IgG Due to PCP Ingestion

Treatment	Total Leucocyte	Gamma Globulin	IgG
Control	194 ± 35	261 ± 37	264 ± 79
5 mg/kg	119 ± 15[b]	89 ± 10[c]	119 ± 11[b]
10 mg/kg	114 ± 6[b]	163 ± 28[b]	111 ± 16[b]
15 mg/kg	106 ± 11[b]	106 ± 14[c]	190 ± 21

[a]Mean ± SE (Day 30/Day 0) determinations of six pigs
[b]Significantly different from controls $p < .05$
[c]Significantly different from controls $p < .01$

In conclusion, after examination of the increases in white blood cells, serum gamma globulin, and serum IgG it was apparant that, relative to the control group, even the lowest dose treatment group (5 mg/kg) was immunologically suppressed (TABLE 1). The sensitivity of these immunological parameters is substantiated by the observation that they were affected when signs indicating overt clinical toxicity were absent (GREICHUS et al., 1979). Even though the total white blood cell values for the treatment groups were still within a normal range (11-32 X $10^3/mm^3$ (KANEKO, 1973)), the fact that, while they were near the lower limits, the control group was able to maintain a more normal concentration indicates some, if only minor leucotoxicity. Likewise, although there was an overall increase in the other parameters investigated, these increases were significantly less for the treatment groups than for the control group. These findings suggest that PCP alone may be toxic for the hematopoietic and/or lymphoid systems. However technical grade PCP, rather than pure PCP, is the most frequently used form of this pesticide. This material is frequently contaminated with chlorodibenzodioxins and chlorodibenzofurans. PARKER et al., (1980) has demonstrated that these contaminants are primarily responsible for the chronic toxicity of commercial grade PCP. A subsequent investigation is therefore being conducted to compare the effects of pure PCP with those of technical grade PCP. In addition to the immunologic parameters reported here, this study will determine the effect of PCP on the various white blood cell populations and on the antibody response to bovine serum albumin.

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